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ORIGINAL RESEARCH

Epidemiology of and risk factors for infection with extended-spectrum β-lactamase-producing carbapenem-resistant Enterobacteriaceae: results of a double case–control study

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Purpose: Carbapenem-resistant *Enterobacteriaceae* (CRE) have been increasingly reported worldwide and pose a serious public threat, but the clinical significance of extended-spectrum β-lactamase (ESBL) production in CRE is not well established.

Patients and methods: A retrospective case–case–control study was conducted to identify the clinical characteristics of patients with ESBL-CRE. The susceptibility of isolates obtained from these patients was assessed. The detection of ESBL and carbapenemase-related genes was performed by PCR methods. Predictors of 30-day mortality in patients with ESBL-CRE infection were also identified in our study.

Results: A total of 149 patients with CRE infection caused by *Enterobacter cloacae* (n=74), *Escherichia coli* (n=38), and *Klebsiella pneumoniae* (n=37) were identified in Chongqing, Southwestern China, between January 2011 and December 2014. Of the 35 isolates detected with carbapenemase-related genes, 16 isolates had New Delhi metallo-β-lactamase (NDM), nine isolates had K. pneumoniae carbapenemase (KPC), seven isolates had imipenemase (IMP), and four isolates had oxacillinase (OXA)-1. One strain of *enterobacter cloacae* carried both NDM-1 and IMP-8 genes. ESBL isolates included the genes CTX-M (72/149), SHV (64/149), and TEM (54/149). All ESBL-CRE isolates exhibited ertapenem resistance, and the rate of cephalosporin resistance was relatively high in general. Independent risk factors for infection with ESBL-CRE included previous exposure to β-lactam antibiotics, transfer from another hospital, and some underlying diseases. In addition, solid tumors, hypoalbuminemia, and central venous catheters were independent predictors of mortality in patients with ESBL-CRE infection.

Conclusion: Physicians should understand the peculiar predictors for the identification of these organisms among high-risk patients.

Keywords: risk factors, carbapenem, resistance, ESBL, mortality

Introduction

In recent years, the emergence and spread of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* have posed a serious threat to public health.[1] ESBL production is often accompanied by other resistance mechanisms that provide cross-resistance to some other antimicrobial agents, such as aminoglycosides and fluoroquinolones.[2] Patients at high risk for ESBL-producing infections often experience greater mortality, longer hospitalization, and higher costs of treatment.[3,4] Carbapenems, potent antibiotics used in treating Gram-negative bacilli infections, are frequently used for suspected or diagnosed infections caused by ESBL-producing bacteria.[5] With the

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increased utility of carbapenem driven by the dissemination of ESBL-producing *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged over the past decade.[6] Notably, the production of ESBL by CRE has been increasingly documented worldwide in recent years.[7,8] This increase is worrisome since most of these isolates harbor both ESBL and carbapenemase genes that confer a higher level of resistance to both carbapenem and cephalosporin. Therefore, the emergence and spread of ESBL-CRE leave few therapeutic options, and the issue of ESBL production in CRE deserves special attention.

Previous studies have analyzed the risk factors in patients with CRE infection, which can be classified as being related to severe comorbid conditions, extensive invasive procedures, or heavy exposure to antibiotics.[9,10] However, few studies have specifically assessed the risk factors and clinical outcomes for carbapenem-resistant ESBL-producing *Enterobacteriaceae* (ESBL-CRE) infection.[11] Moreover, most studies have used the traditional case–control design to identify the risk factors for CRE infection, overestimating the effect of antibiotics by comparing resistant and susceptible isolates.[12,13] The aim of our study was to identify some specific risk factors associated with ESBL-CRE infection by using a case–case–control study. Moreover, predictors for mortality in patients with ESBL-CRE infection were also identified in our study.

Materials and methods

Study setting and study design

This retrospective case–case–control study was performed from January 2011 to December 2014 in the First Affiliated Hospital of Chongqing Medical University, a tertiary university hospital with 3,200 beds in Chongqing, Southwest China. Patients with isolates (including *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli*) that were resistant to at least one carbapenem were enrolled in the study. Patients admitted for <48 hours and those with duplicate isolates were excluded. The three study groups in our analysis were defined as follows: the first case group consisted of patients with ESBL-CRE infection during hospitalization; the second case group consisted of patients with a positive culture for CRE but without ESBL production (non-ESBL-CRE); and the third uninfected control group was randomly selected from among patients hospitalized during the same period of time with a 1:1 ratio to the ESBL-CRE case group, consisting of patients with no clinical cultures positive for *Enterobacteriaceae* during hospitalization.

Antimicrobial susceptibilities and ESBL identification

Bacterial cultures were processed in the clinical microbiology laboratory. Isolates were identified using the VITEK 2 Compact system or the VITEK MS system (bioMérieux, Marcy l'Etoile, Lyon, France) and antimicrobial susceptibilities were determined in vitro using a VITEK 2 Compact AST-GN13 card (bioMérieux). All the carbapenem-resistant isolates (with resistance to at least one of the carbapenems, including imipenem, meropenem, and ertapenem) were confirmed manually by the standard broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[14] *E. coli* American Type Culture Collection (ATCC) 25922 was used as a quality control strain during the antimicrobial susceptibility testing. Additionally, VITEK 2 compact AST-GN13 cards were used to test the antibiotic susceptibilities of all isolates to ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), gentamicin (GM), tobramycin (TOB), ciprofloxacin (CIP), and levofloxacin (LEV).

ESBL production was measured by the double-disk synergy test and the disk diffusion method performed on Mueller-Hinton agar supplemented with cloxacillin (250 mg/L); these tests were interpreted as defined in previously described studies.[2] Additionally, the presence of β-lactamase-encoding genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}) and carbapenemase-encoding genes (bla_{KPC}, bla_{IMP}, bla_{NDM}, and bla_{OXA}) were determined by PCR, as previously described.[7]

Data collection and definitions

Relevant demographics and clinical data of the enrolled patients were extracted from medical records or directly from physicians if needed. The following parameters were used: 1) demographics: age, gender, hospital transfer, intensive care unit (ICU) admission, and 21-day mortality; 2) underlying and concomitant diseases: hypertension, diabetes, solid tumor, hypoproteinemia, hypokalemia, and anemia, as well as respiratory, cardiovascular, liver, renal, and endocrine system diseases; 3) invasive operations before a positive culture: previous surgery in the past 6 months, parenteral nutrition, mechanical ventilation, urinary catheter, drainage tube, gastric tube, tracheal cannula, nasal catheter, bladder irrigation, and central venous catheter within the prior 4 weeks; and 4) source of infection determined as pneumonia, urinary tract infection (UTI), surgical site infection, intra-abdominal infection, or line-related infection using the definitions of the US Centers for Disease Control and Prevention (CDC).[15] Patients ≥60 years old were defined as elderly. Severe anemia was defined as hemoglobin level <60 g/L. Hypoproteinemia was defined as serum total

protein level <60 g/L or albumin level <25 g/L. Hypokalemia was defined as serum potassium level <3.5 mmol/L.

Sample size calculations and statistical analysis

In our study, we assumed that there would be 12% CRE cases vs 88% non-CRE cases.[16] Based on previously published data regarding the non-ESBL isolates, we estimated that non-ESBL-CRE will be 12.8% of cases and non-ESBL control will be 49.9%.[7,17–19] To determine a difference at the 0.05 significance level with 80% power, we estimated that we would need at least 14 non-ESBL-CRE vs 114 non-ESBL control cases (EpiInfo, version 3.3.2).

All analyses were performed using SPSS version 21.0 software (SPSS, Chicago, IL, USA). Univariate analyses were performed separately for each of the variables. Categorical variables were presented as frequencies and percentages and were compared using the McNemar test. Continuous variables were presented as medians and interquartile ranges and were compared using Student's *t*-test (normally distributed variables) and Wilcoxon rank-sum test (nonnormally distributed variables). ORs and 95% CIs were calculated to evaluate the strength of any association. Variables with $P<0.10$ in the univariate analysis were included in the logistic regression model for the multivariate analysis.

Ethics

The study was approved by the Chongqing Medical University Institutional Review Board and Biomedical Ethics Committee. The ethics committee waived the need for written informed consent provided by participants due to the retrospective nature of the study. Because all patient data were analyzed in anonymity, no additional informed consent was required.

Results

Study population

A total of 149 patients with CRE infection caused by *E. cloacae* (n=74), *E. coli* (n=38), and *K. pneumoniae* (n=37) were identified over the 4-year study period. These nonduplicated isolates were mainly cultured from urine (n=48), followed by respiratory tract secretion (n=39), wound exudate (n=31), and blood (n=31). Among these isolates, the numbers that possessed ESBL-related genes were as follows: 65 (43.6%) bla_{CTX-M} genes, 54 (36.2%) bla_{TEM} genes, and 64 (43.0%) bla_{SHV} genes. Moreover, 35 isolates carried carbapenemase-related genes: 16 isolates possessed bla_{NDM}, nine isolates carried bla_{KPC}, seven isolates contained bla_{IMP}, and four isolates had bla_{OXA}. One strain of *enterobacter cloacae* carried both NDM-1 and IMP-8 genes. (Table 1). Of the enrolled patients, 117 with ESBL-CRE (Case I group) and 32 with non-ESBL-CRE (Case II group) were identified. A total of 117 patients without *Enterobacteriaceae* infection served as the control group and were randomly matched to ESBL-CRE cases at a 1:1 ratio. Therefore, 266 patients were included in the final study cohort.

Antimicrobial susceptibility

As shown in Table 2, all isolates were resistant to ertapenem, while only 39.6% (59/149) and 31.5% (47/149) of the isolates were resistant to imipenem and meropenem, respectively. The proportion of isolates that were not sensitive to cephalosporins was relatively high: 91.3%, 88.6%, and 77.2% of the isolates showed no sensitivity to CAZ, CRO, and FEP, respec-

Table 1 The ESBL-related and carbapenemase-related genes of *E. cloacae, E. coli,* and *K. pneumoniae*

Microorganism (number of strains)	ESBL types (number of isolates)			Carbapenemase types (number of isolates)			
	CTX-M	TEM	SHV	KPC	NDM	IMP	OXA
E. cloacae (74)	CTX-M-3 (3)	TEM-1 (32)	SHV-2 (23)	KPC-2 (3)	NDM-1 (7)	IMP-4 (2)	–
	CTX-M-9 (11)	–	–	–	–	IMP-8 (4)	–
	CTX-M-14 (5)	–	–	–	–		–
E. coli (38)	CTX-M-1 (18)	TEM-1 (8)	SHV-2 (6)	KPC-2 (3)	NDM-1 (1)	–	OXA-1 (4)
	CTX-M-3 (3)	–	–	–	NDM-5 (3)	–	–
	CTX-M-9 (5)	–	–	–	–	–	–
	CTX-M-14 (3)	–	–	–	–	–	–
	CTX-M-55 (1)	–	–	–	–	–	–
K. pneumoniae (37)	CTX-M-3 (2)	TEM-1 (14)	SHV-1 (5)	KPC-2 (3)	NDM-1 (5)	IMP-8 (1)	–
	CTX-M-15 (2)	–	SHV-11 (7)	–	–	–	–
	CTX-M-24 (10)	–	SHV-12 (20)	–	–	–	–
	CTX-M-52 (2)	–	SHV-26 (3)	–	–	–	–

Notes: [a]Some data were collected from our previously published studies (Yan et al,[7] Zhang et al,[17] and Jia et al[18]). "–" indicates data not available.
Abbreviation: ESBL, extended-spectrum β-lactamase.

Table 2 The antimicrobial susceptibility of *E. cloacae, E. coli,* and *K. pneumoniae*

Antibiotics	*K. pneumoniae* (n=37)			*E. coli* (n=38)			*E. cloacae* (n=74)		
	S	I	R	S	I	R	S	I	R
Ceftriaxone	7 (18.9)	3 (8.1)	27 (73.0)	1 (2.6)	1 (2.6)	36 (94.8)	3 (4.1)	2 (2.7)	69 (93.2)
Ceftazidime	4 (10.8)	2 (5.4)	31 (83.8)	1 (2.6)	2 (5.3)	35 (92.1)	3 (4.1)	1 (1.4)	70 (94.5)
Cefepime	13 (35.1)	0 (0)	24 (64.9)	5 (13.2)	1 (2.6)	32 (84.2)	13 (17.6)	2 (2.7)	59 (79.7)
Ciprofloxacin	9 (24.3)	6 (16.2)	22 (59.5)	6 (15.8)	0 (0)	32 (84.2)	33 (44.6)	2 (2.7)	39 (52.7)
Levofloxacin	9 (24.3)	3 (8.1)	25 (67.6)	4 (10.5)	0 (0)	34 (89.5)	37 (50.0)	4 (5.4)	33 (44.6)
Gentamycin	14 (37.8)	1 (2.7)	22 (59.5)	7 (18.4)	0 (0)	31 (81.6)	31 (41.9)	4 (5.4)	39 (52.7)
Tobramycin	12 (32.4)	1 (2.7)	24 (64.9)	20 (52.6)	0 (0)	18 (47.4)	29 (39.1)	5 (6.8)	40 (54.1)
Imipenem	14 (37.8)	6 (16.2)	17 (45.9)	20 (52.6)	3 (7.9)	15 (39.5)	42 (56.8)	5 (6.8)	27 (36.5)
Meropenem	16 (43.2)	7 (18.9)	14 (37.8)	28 (73.7)	2 (5.3)	8 (21.1)	45 (60.8)	4 (5.4)	25 (33.8)
Ertapenem	0 (0)	0 (0)	37 (100)	0 (0)	0 (0)	38 (100)	0 (0)	0 (0)	74 (100)

Note: S, susceptible; I, intermediate-resistant; R, resistant.

tively. For fluoroquinolones, 93 (62.4%) and 92 (61.7%) isolates were resistant to CIP and LEV, respectively. Notably, the resistance rates of CIP and LEV were significantly higher in *E. coli* than in *K. pneumoniae* and *E. cloacae* ($P<0.05$). For aminoglycosides, 92 (61.7%) isolates were resistant to GM and 82 (55.0%) were resistant to TOB. Additionally, a total of 55.0% (82/149) of the isolates were classified as multidrug resistant (MDR), including 19 *E. coli* isolates, 22 *K. pneumoniae* isolates, and 41 *E. cloacae* isolates.

Analysis of ESBL-CRE infections vs controls

As shown by the univariate analysis in Table 3, risk factors for ESBL-CRE infection were significantly more frequent in patients with ICU admissions, urinary system disease, concomitant infections, gastric tubes, nasal catheters, central venous catheters, or exposure to cephalosporins and carbapenem. According to the multivariate analysis, urinary system disease (OR: 2.15, 95% CI: 1.03–4.50, $P=0.042$), concomitant infections (OR: 5.29, 95% CI: 1.52–18.41, $P=0.009$), cephalosporin exposure (OR: 7.50, 95% CI: 3.85–14.62, $P<0.001$), and carbapenem exposure (OR: 4.80, 95% CI: 1.56–14.79, $P=0.006$) were identified as independent risk factors for infection with ESBL-CRE when compared with the uninfected controls (Table 4).

Analysis of non-ESBL-CRE infections vs controls

According to the univariate analysis, the risk factors for the acquisition of non-ESBL-CRE were found to be statistically significant for patients who underwent surgery in the past 6 months and had concomitant infections, central venous catheters, and exposure to carbapenem. According to the multivariate analysis, concomitant infections (OR: 4.73, 95% CI: 1.10–20.28, $P=0.036$), central venous catheters (OR: 3.41, 95% CI: 1.26–9.23, $P=0.016$), and exposure to carbapenem (OR: 5.90, 95% CI: 1.56–22.33, $P=0.009$) were identified to be independent risk factors for infection with non-ESBL-CRE when compared with the uninfected controls (Tables 3 and 4).

When comparing risk factors for infection with ESBL-CRE and non-ESBL-CRE relative to controls, we found that concomitant infections and exposure to carbapenem were both associated with the ESBL-CRE and non-ESBL-CRE groups. However, urinary system diseases and exposure to cephalosporins were associated solely with ESBL-CRE infection. Additionally, having a central venous catheter was identified to be a unique risk factor for non-ESBL-CRE infection.

Clinical outcomes: predictors for mortality

During the study period, the overall 21-day mortality rate of all patients was 26.5% (31/117). The results of the univariate and multivariate analyses of risk factors for 30-day mortality are shown in Table 5. The univariate analysis revealed that the presence of solid tumors, hypoproteinemia, tracheal cannula, and central venous catheters resulted in significant differences between the survivor and nonsurvivor groups. According to the multivariate analysis, the predictors independently associated with 30-day mortality were solid tumors (OR: 16.57, 95% CI: 4.22–65.10, $P<0.001$), hypoalbuminemia (OR: 6.06, 95% CI: 1.95–18.80, $P=0.002$), and central venous catheters (OR: 4.20, 95% CI: 1.40–12.62, $P=0.010$).

Discussion

To our knowledge, this case–case–control study is the first analysis to systematically evaluate the risk factors for ESBL-

Table 3 Univariate analysis of risk factors for infection with ESBL-CRE and non-ESBL-CRE

Variables	ESBL-CRE (n=117)	Non-ESBL-CRE (n=32)	Controls (n=117)	ESBL-CRE vs controls		Non-ESBL-CRE vs controls	
				OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Elderly	68 (58.1)	15 (46.9)	57 (48.7)	1.46 (0.87–2.45)	0.149	0.93 (0.42–2.03)	0.853
Male gender	64 (54.7)	17 (53.1)	51 (43.6)	1.56 (0.93–2.62)	0.089	1.47 (0.67–3.21)	0.337
Transferring from another hospital	25 (21.4)	9 (28.1)	22 (18.8)	1.17 (0.62–2.23)	0.624	1.69 (0.68–4.15)	0.250
ICU admission	52 (44.4)	6 (18.8)	35 (29.9)	1.87 (1.09–3.21)	**0.021**	0.54 (0.21–1.43)	0.210
Acute and chronic conditions on admission							
Hypertension	43 (36.8)	14 (43.8)	33 (28.2)	1.48 (0.85–2.57)	0.163	1.98 (0.88–4.43)	0.094
Diabetes	22 (18.8)	7 (21.9)	23 (19.7)	0.95 (0.49–1.81)	0.868	1.14 (0.44–2.97)	0.782
Solid tumor	19 (16.2)	7 (21.9)	17 (14.5)	1.14 (0.56–2.32)	0.717	1.65 (0.62–4.40)	0.317
Respiratory disease	36 (30.8)	12 (37.5)	30 (25.6)	1.29 (0.73–2.28)	0.383	1.74 (0.76–3.98)	0.186
Hepatobiliary disease	26 (22.2)	6 (18.8)	20 (17.1)	1.39 (0.72–2.65)	0.324	1.12 (0.41–3.07)	0.827
Gastrointestinal diseases	16 (13.7)	6 (18.8)	19 (16.2)	0.82 (0.40–1.68)	0.582	1.19 (0.43–3.28)	0.736
Chronic kidney disease	33 (28.2)	11 (34.4)	30 (25.6)	1.14 (0.64–2.03)	0.658	1.52 (0.66–3.52)	0.327
Urinary system diseases	39 (33.3)	8 (25.0)	22 (18.8)	2.16 (1.18–3.94)	**0.011**	1.44 (0.57–3.63)	0.439
Cardiovascular disease	17 (14.5)	3 (9.4)	10 (8.5)	1.82 (0.80–4.16)	0.152	1.11 (0.29–4.29)	0.883
Immune system disease	11 (9.4)	3 (9.4)	9 (7.7)	1.25 (0.50–3.13)	0.640	1.24 (0.32–4.88)	0.757
Hypoalbuminemia	52 (44.4)	12 (37.5)	43 (36.8)	1.38 (0.82–2.32)	0.231	1.03 (0.46–2.32)	0.938
Severe anemia	3 (2.6)	2 (6.3)	6 (5.1)	0.49 (0.12–1.99)	0.308	1.23 (0.24–6.42)	0.803
Hypokalemia	29 (24.8)	9 (28.1)	21 (17.9)	1.51 (0.80–2.83)	0.202	1.79 (0.72–4.42)	0.203
Urinary tract infection	29 (24.8)	6 (18.8)	18 (15.4)	1.81 (0.94–3.49)	0.073	1.27 (0.46–3.52)	0.646
Pulmonary infection	29 (24.8)	5 (15.6)	19 (16.2)	1.70 (0.89–3.24)	0.105	0.96 (0.33–2.79)	0.933
Intra-abdominal infection	7 (6.0)	2 (6.3)	2 (1.7)	3.66 (0.74–18.00)	0.089	3.83 (0.52–28.35)	0.159
Concomitant infection	20 (17.1)	6 (18.8)	4 (3.4)	5.83 (1.93–17.63)	**0.001**	6.52 (1.72–24.77)	**0.002**
Invasive procedures within prior 4 weeks							
Parenteral nutrition	12 (10.3)	5 (15.6)	8 (6.8)	1.56 (0.61–3.96)	0.350	2.52 (0.76–8.33)	0.119
Mechanical ventilation	20 (17.1)	6 (18.8)	13 (11.1)	1.65 (0.78–3.50)	0.189	1.85 (0.64–5.32)	0.251
Urinary catheter	51 (43.6)	14 (43.8)	40 (34.2)	1.49 (0.88–2.52)	0.140	1.50 (0.68–3.32)	0.319
Drainage tube	44 (37.6)	12 (37.5)	34 (29.1)	1.47 (0.85–2.54)	0.166	1.47 (0.65–3.32)	0.360
Gastric tube	31 (26.5)	7 (21.9)	17 (14.5)	2.12 (1.10–4.10)	**0.023**	1.65 (0.62–4.40)	0.317
Trachea cannula	20 (17.1)	8 (25.0)	19 (16.2)	1.06 (0.53–2.12)	0.861	1.72 (0.67–4.40)	0.254
Nasal catheter	22 (18.8)	2 (6.3)	10 (8.5)	2.48 (1.12–5.50)	**0.022**	0.71 (0.15–3.43)	0.672
Bladder irrigation	13 (11.1)	4 (12.5)	8 (6.8)	1.70 (0.68–4.28)	0.253	1.95 (0.55–6.93)	0.297
Central venous catheter	30 (25.6)	12 (37.5)	16 (13.7)	2.18 (1.11–4.26)	**0.021**	3.79 (1.56–9.21)	**0.002**
Surgery in the past 6 months	56 (47.9)	19 (59.4)	46 (39.3)	1.19 (0.71–1.99)	0.512	2.26 (1.02–5.01)	**0.043**
Antimicrobial exposure within 3 months							
Cephalosporins	82 (70.1)	11 (34.4)	25 (21.4)	8.62 (4.76–15.61)	<0.001	1.93 (0.82–4.52)	0.128
Carbapenem	31 (26.5)	7 (21.9)	5 (4.3)	8.07 (3.01–21.63)	<0.001	6.27 (1.84–21.39)	**0.001**
Aminoglycosides	16 (13.7)	4 (12.5)	14 (12.0)	1.17 (0.54–2.51)	0.696	1.05 (0.32–3.44)	0.935
Quinolones	27 (23.1)	3 (9.4)	22 (18.8)	1.30 (0.69–2.44)	0.422	0.45 (0.12–1.60)	0.206
Tetracyclines	6 (5.1)	1 (3.1)	3 (2.6)	2.05 (0.5–8.42)	0.308	1.23 (0.12–12.20)	0.862
Macrolides	4 (3.4)	3 (9.4)	4 (3.4)	1.00 (0.24–4.1)	1.000	2.92 (0.62–13.79)	0.158
Metronidazole	26 (22.2)	7 (21.9)	33 (28.2)	0.73 (0.4–1.32)	0.292	0.71 (0.28–1.81)	0.474
Glycopeptides	18 (15.4)	5 (15.6)	14 (12.0)	1.34 (0.63–2.83)	0.447	1.36 (0.45–4.12)	0.582

Note: Values are presented as n (%), unless otherwise noted. Bold face indicates values that are significant ($P<0.05$).
Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; ICU, intensive care unit.

CRE infection and the predictors of mortality. In this work, we identified several particularly important findings. First, the most frequent ESBL-CRE species observed in our study was *E. cloacae*, followed by *E. coli* and *K. pneumoniae*. This distribution was notably different from that of other studies on CRE carried out in the USA and Europe.[20,21] Among the CRE isolates carrying ESBL genes, CTX-M was the most prevalent type in *E. coli*, revealing that CTX-M *E. coli* isolates are widely spread among ESBL-CRE isolates in our region.

Second, we reported for the first time that urinary system disease is an independent predictor associated with the isolation of ESBL-CRE. One explanation for this finding is that many patients with urinary obstruction or incontinence

Table 4 Multivariate analysis of risk factors for infection with ESBL-CRE and non-ESBL-CRE

Variables	ESBL-CRE vs controls			Non-ESBL-CRE vs controls		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Urinary system diseases	2.15	1.03–4.50	0.042	–	–	–
Concomitant infection	5.29	1.52–18.41	0.009	4.73	1.10–20.28	0.036
Central venous catheter	–	–	–	3.41	1.26–9.23	0.016
Cephalosporins	7.50	3.85–14.62	<0.001	–	–	–
Carbapenems	4.80	1.56–14.79	0.006	5.90	1.56–22.33	0.009

Note: "–" indicates data not available.
Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae;* ESBL, extended-spectrum β-lactamase.

Table 5 Risk factors associated with 30-day mortality

Variables	Nonsurvivors (n=31)	Survivors (n=86)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Elderly	20 (64.5)	48 (55.8)	1.44 (0.62–3.37)	0.400	–	–
Male gender	18 (58.1)	46 (53.5)	1.20 (0.53–2.76)	0.661	–	–
Transferring from another hospital	8 (25.8)	17 (19.8)	1.41 (0.54–3.70)	0.482	–	–
ICU admission	17 (54.8)	35 (40.7)	1.77 (0.77–4.05)	0.174	–	–
Solid tumor	13 (41.9)	6 (7.0)	9.63 (3.22–28.76)	<0.001	16.57 (4.22–65.10)	<0.001
Respiratory disease	13 (41.9)	23 (26.7)	1.98 (0.84–4.67)	0.116	–	–
Chronic kidney disease	10 (32.3)	23 (26.7)	1.30 (0.54–3.18)	0.559	–	–
Urinary system diseases	10 (32.3)	29 (33.7)	0.94 (0.39–2.25)	0.882	–	–
Surgery in the past 6 months	19 (61.3)	37 (43.0)	2.10 (0.91–4.85)	0.081	–	–
Hypoalbuminemia	23 (74.2)	9 (10.5)	5.65 (2.25–14.19)	<0.001	6.06 (1.95–18.80)	**0.002**
Hypokalemia	8 (25.8)	21 (24.4)	1.08 (0.42–2.76)	0.878	–	–
Parenteral nutrition	4 (12.9)	8 (9.3)	1.44 (0.40–5.18)	0.571	–	–
Trachea cannula	9 (29.0)	11 (12.8)	2.79 (1.03–7.59)	**0.039**	–	–
Central venous catheter	14 (45.2)	16 (18.6)	3.60 (1.48–8.79)	**0.004**	4.20 (1.40–12.62)	**0.010**
Cephalosporins	23 (74.2)	59 (68.6)	1.32 (0.52–3.32)	0.560	–	–
Carbapenem	7 (22.6)	24 (27.9)	0.75 (0.29–1.98)	0.565	–	–
Aminoglycosides	3 (9.7)	13 (15.1)	0.60 (0.16–2.27)	0.450	–	–
Quinolones	5 (16.1)	22 (25.6)	0.56 (0.19–1.64)	0.284	–	–
Tetracyclines	1 (3.2)	5 (5.8)	0.54 (0.06–4.81)	0.575	–	–
Macrolides	1 (3.2)	3 (3.5)	0.92 (0.09–9.21)	0.945	–	–
Concomitant infection	4 (12.9)	16 (18.6)	0.65 (0.20–2.11)	0.470	–	–

Notes: Values are presented as n (%), unless otherwise noted. Bold face indicates values that are significant ($P<0.05$). "–" indicates data not available.
Abbreviation: ICU, intensive care unit.

require some implanted medical devices, such as a urinary catheter, or suprapubic cystostomy, increasing the possibility of bacterial adherence, biofilm formation, and some morphological changes.[22] Furthermore, patients with symptomatic UTIs are usually treated with prolonged or multiple antibiotic exposures, which may lead to long-term changes in the normal microbiota of the gastrointestinal tract and the development of MDR microorganisms.[23]

Third, we observed that central venous catheters were identified to be independently associated with non-ESBL-CRE, and this association was well established by a previous study on CRE.[24] Compared with previous observations, our results revealed that carbapenem exposure and concomitant infections are common risk factors for infection with ESBL-CRE and non-ESBL-CRE, demonstrating that these factors may be associated with CRE infection in general. First, possibly due to the increased and inappropriate use of carbapenem, selective pressure exerted by these agents could potentially promote the emergence and spread of CRE in China. Second, dysbacteriosis induced by large doses of antibiotics could stimulate the development of secondary infections, namely, concomitant infections. Third, most of these patients with concomitant infections have more severe underlying diseases and lower immunity, which may make them more vulnerable to acquiring CRE infection.

Fourth, solid tumors, hypoalbuminemia, and central venous catheters were linked to a significantly increased risk of mortality. Many cancer patients infected with resistant bacteria often receive inappropriate initial antimicrobial therapy, which may impair outcomes, prolong hospitalization,

and increase mortality.[25] Moreover, hypoalbuminemia, as an acute phase response, may have a strong predictive value for the severity of underlying conditions, as it is the main cause of increased mortality in some malnourished patients.[26] In addition, some invasive devices that patients receive may destroy intestinal barrier functions, promote formation of microbial biofilms, and possibly lead to catheter-related bloodstream infections, thus increasing mortality in these patients.[27]

Our study has several limitations. First, this retrospective study was conducted at a single medical center, and our sample size was relatively small. Therefore, our findings might not be generalizable to other multicenter studies. Second, the clonality of the resistant isolates at the molecular level was not examined in our study. Therefore, potential outbreaks might not be ruled out. Finally, our study focused only on clinically significant ESBL-CRE strains, which underestimates the burden of colonizing CRE isolates with ESBL production.

Conclusion

This case–case–control study was conducted retrospectively to assess the clinical predictors associated with ESBL-CRE and non-ESBL-CRE. Our findings differed from those of previous studies, showing that urinary system disease could be independently associated with the isolation of ESBL-CRE. Moreover, another important finding of this study is that carbapenem exposure and concomitant infections are common risk factors for infection with both ESBL-CRE and non-ESBL-CRE. We also identified some peculiar factors that could have deleterious effects on clinical outcomes. Therefore, effective control measures and standard antibiotic stewardship efforts should be taken up to prevent the further spread of ESBL-CRE strains within different hospitals.

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Author contributions

All authors contributed toward data analysis, drafting, and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Tansarli GS, Poulikakos P, Kapaskelis A, Falagas ME. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: evaluation of the evidence--systematic review. *J Antimicrob Chemother*. 2014;69(5):1177–1184.
2. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev*. 2005;18(4):657–686.
3. Tumbarello M, Sanguinetti M, Montuori E, et al. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. *Antimicrob Agents Chemother*. 2007;51(6):1987–1994.
4. Maslikowska JA, Walker SA, Elligsen M, et al. Impact of infection with extended-spectrum β-lactamase-producing Escherichia coli or Klebsiella species on outcome and hospitalization costs. *J Hosp Infect*. 2016;92(1):33–41.
5. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing Enterobacteriaceae. *Clin Microbiol Rev*. 2018;31(2).
6. Palmore TN, Henderson DK. Carbapenem-resistant Enterobacteriaceae: a call for cultural change. *Ann Intern Med*. 2014;160(8):567–569.
7. Yan J, Pu S, Jia X, et al. Multidrug Resistance Mechanisms of Carbapenem Resistant Klebsiella pneumoniae Strains Isolated in Chongqing, China. *Ann Lab Med*. 2017;37(5):398–407.
8. Eser OK, Altun Uludağ H, Ergin A, Boral B, Sener B, Hasçelik G. Carbapenem resistance in ESBL positive Enterobacteriaceae isolates causing invasive infections. *Mikrobiyol Bul*. 2014;48(1):59–69.
9. Zhao ZC, Xu XH, Liu MB, Wu J, Lin J, Li B. Fecal carriage of carbapenem-resistant Enterobacteriaceae in a Chinese university hospital. *Am J Infect Control*. 2014;42(5):e61–e64.
10. Freeman R, Moore LS, Charlett A, Donaldson H, Holmes AH. Exploring the epidemiology of carbapenem-resistant Gram-negative bacteria in west London and the utility of routinely collected hospital microbiology data. *J Antimicrob Chemother*. 2015;70(4):1212–1218.
11. Kritsotakis EI, Tsioutis C, Roumbelaki M, Christidou A, Gikas A. Antibiotic use and the risk of carbapenem-resistant extended-spectrum-{beta}-lactamase-producing Klebsiella pneumoniae infection in hospitalized patients: results of a double case-control study. *J Antimicrob Chemother*. 2011;66(6):1383–1391.
12. Bart Y, Paul M, Eluk O, Geffen Y, Rabino G, Hussein K. Risk Factors for Recurrence of Carbapenem-Resistant Enterobacteriaceae Carriage: Case-Control Study. *Infect Control Hosp Epidemiol*. 2015;36(8):936–941.
13. Ling ML, Tee YM, Tan SG, et al. Risk factors for acquisition of carbapenem resistant Enterobacteriaceae in an acute tertiary care hospital in Singapore. *Antimicrob Resist Infect Control*. 2015;4:26.
14. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. 25th Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
15. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrob Agents Chemother*. 2005;49(2):760–766.
16. Fupin HU, Demei ZHU, Fu W, et al. Report of CHINET Antimicrobial Resistance Surveillance Program in 2015. *Chin J Infect Chemother*. 2016;16:685–694.
17. Zhang C, Xu X, Pu S, et al. Characterization of carbapenemases, extended spectrum β-lactamases, quinolone resistance and aminoglycoside resistance determinants in carbapenem-non-susceptible Escherichia coli from a teaching hospital in Chongqing, Southwest China. *Infect Genet Evol*. 2014;27:271–276.
18. Jia X, Dai W, Ma W, et al. Carbapenem-Resistant *E. cloacae* in Southwest China: Molecular Analysis of Resistance and Risk Factors for Infections Caused by NDM-1-Producers. *Front Microbiol*. 2018;9:658.

19. Fupin HU, Demei ZHU, Fu W, et al. CHINET Surveillance of bacterial resistance in China. *Chin J Infect Chemother*. 2011;2012(12):321–329.
20. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. *Lancet Infect Dis*. 2009;9(4):228–236.
21. Oteo J, Saez D, Bautista V, et al. Carbapenemase-producing enterobacteriaceae in Spain in 2012. *Antimicrob Agents Chemother*. 2013;57(12):6344–6347.
22. Wragg R, Harris A, Patel M, Robb A, Chandran H, Mccarthy L. Extended spectrum beta lactamase (ESBL) producing bacteria urinary tract infections and complex pediatric urology. *J Pediatr Surg*. 2017;52(2):286–288.
23. Kostakioti M, Hultgren SJ, Hadjifrangiskou M. Molecular blueprint of uropathogenic *Escherichia coli* virulence provides clues toward the development of anti-virulence therapeutics. *Virulence*. 2012;3(7):592–593.
24. Hyle EP, Ferraro MJ, Silver M, Lee H, Hooper DC. Ertapenem-resistant Enterobacteriaceae: risk factors for acquisition and outcomes. *Infect Control Hosp Epidemiol*. 2010;31(12):1242–1249.
25. Gudiol C, Carratalà J. Antibiotic resistance in cancer patients. *Expert Rev Anti Infect Ther*. 2014;12(8):1003–1016.
26. Gatta A, Verardo A, Bolognesi M. Hypoalbuminemia. *Intern Emerg Med*. 2012;7(Suppl 3):S193–199.
27. Yousif A, Jamal MA, Raad I. Biofilm-based central line-associated bloodstream infections. *Adv Exp Med Biol*. 2015;830:157–179.